Identification of novel pharmacological modulators of the TMEM16A chloride channel

Other

IDENTIFICATION OF NOVEL PHARMACOLOGICAL MODULATORS OF THE TMEM16A CHLORIDE CHANNEL

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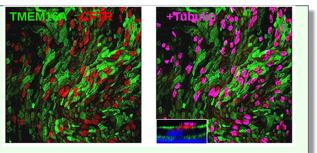
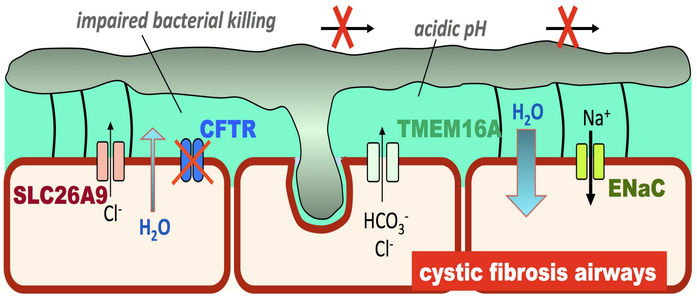
Background – TMEM16A is the protein responsible for Ca^{2+}-activated chloride secretion in airway epithelial cells. Its role in the physiology of the airway epithelium and its suitability as a therapeutic target in cystic fibrosis are still unclear. Actually, TMEM16A expression, in contrast to CFTR, is mostly detected in non-ciliated cells. Pharmacological modulators of TMEM16A could be useful as research tools and as novel therapeutics agents.



AIM: Identification of novel pharmacological modulators of TMEM16A as tools of research and possible therapeutic agents by High Throughput Screening (HTS)

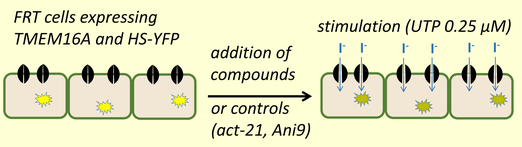


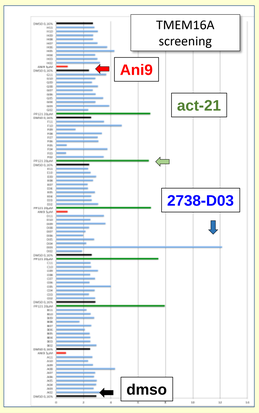
Pharmacological stimulation of TMEM16A is a possible therapeutic strategy to bypass defective Cl- secretion in cystic fibrosis due to CFTR chloride channel defect. Our goal is the identification of novel pharmacological modulators of TMEM16A as tools of research and possible therapeutic agents.

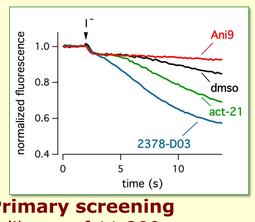
Abstract

TMEM16A is a calcium-activated chloride channel expressed in airway epithelial cells, particularly under conditions of mucus hypersecretion. Activation of TMEM16A by calcium agonists results in enhanced chloride and bicarbonate secretion. Our aim is to identify novel pharmacological modulators of TMEM16A function. Using the halide-sensitive yellow fluorescent protein (HS-YFP) functional assay, we screened a maximally-diverse chemical library (11,300 compounds). For this purpose, we used FRT cells co-expressing TMEM16A and HS-YFP. Cells were stimulated with a low concentration of UTP to induce partial activation of TMEM16A. TMEM16A potentiators and inhibitors are detected as compounds that accelerate or slow down the rate of HS-YFP quenching, respectively. Controls during the screening included the Ani9 inhibitor (Seo et al., PLoS One 11:e0155771, 2016) and a TMEM16A potentiator that we identified in a previous study. After screening the whole library in duplicate, we found 24 compounds that increase TMEM16A activity above a threshold equal to 50% of the positive control. These hits are evaluated in secondary assays based on: i) null FRT cells, to rule out compounds acting on other channels/transporters; ii) stimulation of TMEM16A with ionomycin, to bypass purinergic receptors; iii) evaluation of intracellular calcium mobilization with a fluorescent probe, to detect compounds with an indirect mechanism of action. Compounds with the best characteristics will be tested in CF human bronchial epithelial cells (short-circuit current experiments) to assess the effect on calcium-dependent chloride secretion. The screening also identified possible inhibitors of TMEM16A. Novel pharmacological modulators of TMEM16A (potentiators and inhibitors) could be useful as tools of research and as possible therapeutic agents to improve mucociliary function in CF and other respiratory diseases.

Novel TMEM16A modulators by HTS

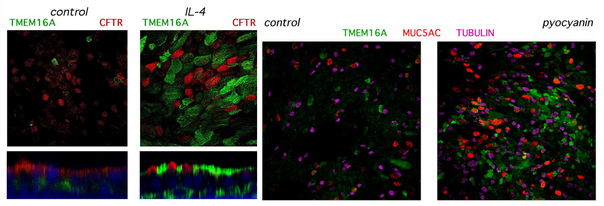






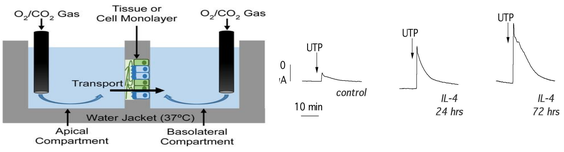
Primary screening

A library of 11,300 compounds was screened with the HS-YFP functional assay. TMEM16A potentiators are identified as compounds that enhance the quenching of YFP fluorescence (effect of UTP). Act-21 and Ani9 are included in each microplate as controls.



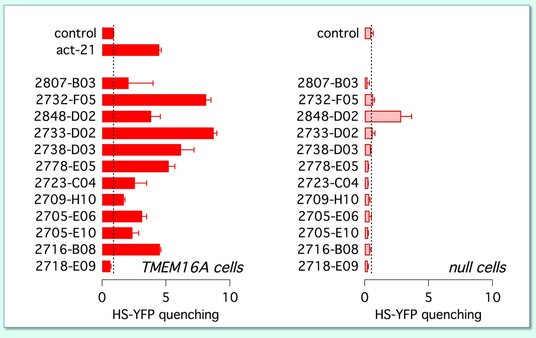
TMEM16A is particularly expressed and active under conditions of mucus metaplasia. Expression of TMEM16A is particularly induced by IL-4/IL-13 or by bacterial components (e.g. pyocyanin) in non-ciliated MUC5AC-positive cells.

Vertical Ussing Chamber

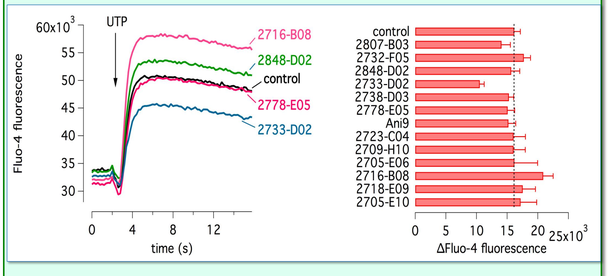


short-circuit current experiments (Isc) experiments are used to measure the transport of chloride as current.

Validation of hits from screening by YFP assay



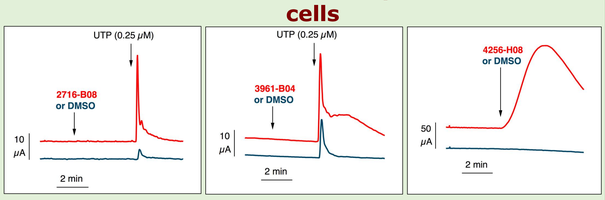
Hits from the primary screening were retested on TMEM16A-expressing and null cells. Except for one compound (2848-D02), all other hits were inactive on null cells.



Fluo-4 is a labeled calcium indicators are molecules that exhibit an increase in fluorescence upon binding Ca^{2+}. It has been used to image the spatial dynamics of Ca^{2+} signaling, in flow cytometry experiments involving photoactivation of caged chelators, second messengers, and neurotransmitters, and for cell-based pharmacological screening.

Hits were also evaluated with the Fluo-4 assay to exclude compounds altering intracellular calcium.

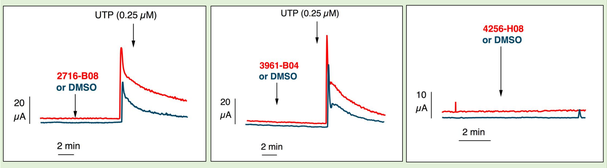
Evaluation of hits with Isc experiments on FRT cells



Active compounds can be grouped in two classes:

- **potentiators of UTP response**
- **activators in the absence of agonist**

Hit validation is in progress on bronchial epithelial cells



So far confirmed hits belong to the class of potentiators, whereas direct activators were found to be inactive in bronchial cells.